Docket No.: 65689CPDV(43382)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Ian Hunter *et al.*

Application No.: 10/820,679 | Confirmation No.: 8534

Filed: April 8, 2004 | Art Unit: 1797

For: METHODS OF LOADING LIQUID SAMPLES INTO THROUGH-HOLE ARRAYS **[AMENDED]** | Examiner: A. Soderquist

**AMENDMENT AND RESPONSE TO ADVISORY ACTION**

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

**INTRODUCTORY COMMENTS**

In response to the Advisory Action dated July 9, 2009 maintaining the objections and rejections of the Office Action of May 5, 2009, please amend the above-identified U.S. patent application as follows:

**Amendments to the Specification** begin on page 2 of this paper.

**Amendments to the Claims** are reflected in the listing of claims which begins on page 5 of this paper.

**Amendments to the Drawings** begin on page 9 of this paper.

**Remarks/Arguments** begin on page 10 of this paper.

## AMENDMENTS TO THE SPECIFICATION

Please amend the Title as follows:

~~REFORMATTED~~ METHODS OF LOADING LIQUID SAMPLES INTO THROUGH-HOLE ARRAYS

Please amend the paragraph spanning from page 12, line 21 to page 13, line 12 of the specification (paragraph [0060] as published) as follows:

As an example of an application in which a genetic library is screened for improved enzymes using a one step assay, an E. coli genetic library is prepared containing mutations in the beta galactosidase enzyme. The E. coli cells are grown to a density in phosphate-limited media such that there is an average of 1 cell for every 200 nl of liquid. The media also contains, MUG, a fluorogenic substrate for beta-galactosidase. A through-hole sheet is prepared with a hydrophobic exterior and hydrophilic through-holes at a density of $10^7$ ~~10A7~~ per square meter. Registration holes are includes in the tape to aid in precise dispensing. Each through-hole holds 70 nl of fluid. A spool of the through-hole sheet is unwound and guided through a trough containing the cell solution, so that each through hole is filled. The sheet is then wound onto spacers and into a receiving spool. The spacers prevent smearing of the liquid and provide for gas transfer in and out of the spool. The spool is incubated in a humidified environment ~~enviroment~~ at 37° C. for 24 hours. The spool is then unwound as it is passed between a uniform photo-illumination source with a wavelength of 350 nm and a CCD imaging system with a 450 nm filter. The position in the sheet of colonies with exceptionally high enzyme activity is recorded and those colonies are retrieved using a robotic microfluid handling system for further analysis. This assay can also be

performed by co-registering and mating a second, identical, sheet containing the fluorogenic substrate with the first sheet containing the bacteria. An absorbance measurement may also be performed to normalize the signal output for the number of bacteria.

Please amend the paragraph spanning from page 19, lines 20-29 of the specification (paragraph [0081] as published) as follows:

In accordance with other embodiments of the present invention, the surface chemistry of the array faces may also be selectively modified by substituting other silanizing agents for the polydimethylsiloxane telomer. This method may advantageously prevent aqueous solutions from adhering to array faces during array loading and also act as physical barriers between the aqueous solutions in adjacent through-holes. During this process a positive pressure of inert gas is applied to the opposite side of the array. The positive pressure within the through-holes prevents the silanizing vapor from reaching the interior surfaces. This method advantageously allows removal and reapplication ~~reaplication~~ of the hydrophobic coating.

Please amend the paragraph spanning from page 23, line 28 to page 24, line 22 of the specification (paragraph [0099] as published) as follows:

Embodiments of the invention also provide for methods for filling a stack of arrays, as shown in cross-section in FIG. 11 , by bringing the end of a tubing array 130 into close proximity with a matching set of through-holes in the array stack 122 . The tubing array can be aligned relative to the array stack by an alignment plate 128 with through-holes having the same center-to-center spacing as the through-holes into which fluid is

placed. Each array 10 in the stack 122 is spaced a small distance s that may be, but is not limited to, an equal distance to the through-hole spacing. Application of pressure to the end of the tubing array, placed inside a pressurized container 132 , forces fluid from each capillary tube 102 into the opposing through-hole. After the through-hole is filled, a liquid drop can begin to grow in the space between the two plates. When the drop reaches a size that it contacts the through-hole in the plate above it, surface tension draws some fluid into the through-hole. Once the fluidic bridge is established, liquid can flow into the through-hole, driven by the constant pressure applied to the opposite end of the tubing array. With no applied pressure, the drop retreats into the through-hole, the fluidic bridge between each plate is broken, and the separation of array plates after filling can be facilitated (i.e., because there is generally no surface tension that needs to be overcome). Successive filled plates 10 are then withdrawn, and the tube array may be retracted in direction 127 ~~128~~. Each vertically registered set of through-holes may thus act as a channel for fluid flow. The hydrophobic coatings on the exterior surface of the arrays prevent liquid from flowing into adjacent holes. This technique can also advantageously be used to create replica plates of a cell library by applying a cell suspension with a pressure uniformly to the array stack.